UN
SECU

AD-A212 502

MENTATION PAGE

Form Approved OMB No. 0704-0188

1a. Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Walter Reed Army Institute of Research	6b. OFFICE SYMBOL (If applicable): SGRD-UWM-A
7a. NAME OF MONITORING ORGANIZATION: U.S. Army Medical Research and Development Command	
6c. ADDRESS (City, State, and ZIP Code): Washington, DC 20307-5100	7b. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5012
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: USAMRDC	8b. OFFICE SYMBOL (If applicable): SGRD-PLA
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
(U) Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives. 2. Stereospecificity of the Ether Side Chain

12. PERSONAL AUTHOR(S)
Lin, A.J.; Lee, M.; and Klayman, D.L.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Manuscript	FROM _____ TO _____	April 1989	5

16. SUPPLEMENTARY NOTATION
Manuscript for publication in Journal of Medicinal Chemistry

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A new series of hydrolytically stable and water-soluble dihydroartemisinin derivatives with optically active side chains was prepared as potential antimalarial agents. This was an effort to prepare compounds with activity superior to that of artelinic acid and to examine the impact of the stereospecificity of the introduced alkyl side chain on biological properties. The ester derivatives (6a-d) possess superior in vitro activity to artemisinin, artemether, and arteether against two strains of Plasmodium falciparum (D-6 and W-2); however, conversion of the esters to their corresponding acids drastically reduces their antimalarial activity. None of the new acids possess in vitro antimalarial activity superior to that of artelinic acid. Although there appears to be limited stereospecificity for antimalarial activity among the acids (7a-d) tested, significant differences in antimalarial activity was seen among the esters.

20. DISTRIBUTION AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
COL David E. Davidson, Jr., VC	(301) 427-5411	SGRD-UWM

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

Published in Journal of Medicinal Chemistry, June 1989, pp. 1249–1252, by the American Chemical Society

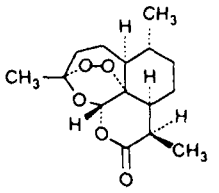
Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives. 2.[1,2] Stereospecificity of the Ether Side Chain

Ai J. Lin,* Margaret Lee, and Daniel L. Klayman

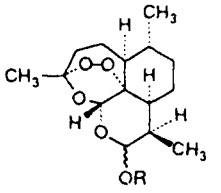
Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.
Received September 6, 1988

A new series of hydrolytically stable and water-soluble dihydroartemisinin derivatives with optically active side chains was prepared as potential antimalarial agents. This was an effort to prepare compounds with activity superior to that of artelinic acid and to examine the impact of the stereospecificity of the introduced alkyl side chain on biological properties. The ester derivatives (**6a–d**) possess superior in vitro activity to artemisinin, artemether, and arteether against two strains of *Plasmodium falciparum* (D-6 and W-2); however, conversion of the esters to their corresponding acids drastically reduces their antimalarial activity. None of the new acids possess in vitro antimalarial activity superior to that of artelinic acid. Although there appears to be limited stereospecificity for antimalarial activity among the acids (**7a–d**) tested, significant differences in antimalarial activity was seen among the esters.

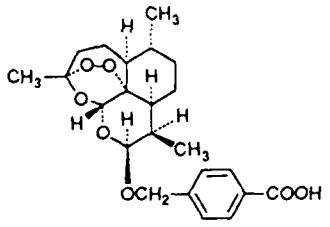
Artemisinin (qinghaosu, **1**), a new clinically valuable antimalarial agent isolated from the plant *Artemisia annua*, is an unusual epidioxide containing sesquiterpene lactone.[3-9] The lactol or hemiacetal form of the sesquiterpene, dihydroartemisinin (**2a**), is also a potent, albeit relatively unstable, antimalarial.[7b] Because the utility of artemisinin as an antimalarial agent[5-7,10-15] is limited by its low solubility in both oil and water, the sesquiterpene has been structurally modified by the formation of short-chain ether derivatives of **2a**, such as artemether (**2b**)[16-22] and arteether (**2c**).[7b,23] Both of these derivatives possess superior lipid solubility and antimalarial activities to the parent artemisinin.[7e]



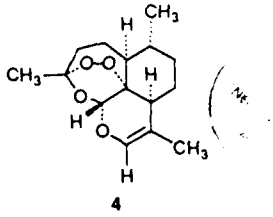
1



2a: R = H (α + β)
b: R = CH_3 (β)
c: R = CH_2CH_3 (β)
d: R = $C(O)CH_2CH_2COOH$ (α)



3

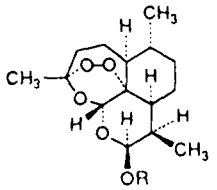


4

A water-soluble derivative of artemisinin, the sodium salt of artesunic acid (**2d**, the succinic acid half-ester derivative of dihydroartemisinin), can be administered by intravenous injection, a property that makes it especially useful in the treatment of advanced and potentially lethal cases of *Plasmodium falciparum*.[7c,12,24,25] Compound **2d** is capable of rapidly reversing parasitemia and causing the restoration to consciousness of the comatose cerebral malaria patient. The utility of sodium artesunate, however, is impaired by its poor stability in aqueous solution due to the facile hydrolysis of the ester linkage.[26] We have reported[2] on a series of new water-soluble derivatives in which the solubilizing group, carboxylate, is on a moiety that is joined to dihydroartemisinin by an ether, rather than an ester, linkage. One of these derivatives, artelinic acid (**3**), is not only considerably more stable than artesunic acid in weakly alkaline solution but is also more active

(1) Presented in part at The First Princess Chulabhorn Science Congress 1987: International Congress on Natural Products, Dec 10–13, 1987, Bangkok, Thailand; Abstract No. AC-25.
(2) Lin, A. J.; Klayman, D. L.; Milhous, W. K. *J. Med. Chem.* **1987**, *30*, 2147.
(3) Liu, J.; Ni, M.; Fan, J.; Tu, Y.; Wu, Z.; Qu, Y.; Chou, W. *Acta Chim. Sinica* **1979**, *37*, 129.
(4) China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials, *J. Tradit. Chin. Med.* **1982**, *2*, 3.
(5) Qinghaosu Research Group, *Scientia Sinica* **1980**, *23*, 380.
(6) Qinghaosu Antimalaria Coordinating Research Group, *Chinese Med. J.* **1979**, *92*, 811.
(7) (a) China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials, *J. Tradit. Chin. Med.* **1982**, *2*, 17. (b) *Ibid.* **1982**, *2*, 9. (c) *Ibid.* **1982**, *2*, 45. (d) Li, G.; Guo, X.; Jin, R.; Jian, H.; Li, Z. *Ibid.* **1982**, *2*, 125. (e) Luo, X.; Shen, C. *Med. Res. Rev.* **1987**, *7* (1), 29.
(8) Klayman, D. L.; Lin, A. J.; Acton, N.; Scovill, J. P.; Hoch, J. M.; Milhous, W. K.; Theoharides, A. D. *J. Nat. Prod.* **1984**, *47*, 715.
(9) (a) Lin, A. J.; Klayman, D. L.; Hoch, J. M.; Silverton, J. V.; George, C. F. *J. Org. Chem.* **1985**, *50*, 4504. (b) Lin, A. J.; Theoharides, A. D.; Klayman, D. L. *Tetrahedron* **1986**, *42*, 2181. (c) Klayman, D. L. *Science (Washington, D.C.)* **1985**, *228*, 1049.
(10) Antimalarial Group of the First and Second Affiliated Hospital of Kungming, *J. New Med.* **1979**, 49.
(11) Bruce-Chwatt, L. *J. Br. Med. J. Clin. Res.* **1982**, *284*, 767.
(12) Department of New Medicine, People's Hospital of Dongfang County, Guangdong *Xinyiyaoxue Zazhi* **1979**, *1*, 20.
(13) Li, G.-Q.; Guo, X.-B.; Jian, H.-X.; Fu, L.-C.; Shen, L.-C.; Li, R.-S.; Dai, B.-Q.; Li, Z.-L. *J. Tradit. Chin. Med.* **1984**, *25*, 26; **1985**, *5*, 159.
(14) Li, Y.; et al. *J. Parasitol. Parasitic Dis.* **1984**, *2*, 279.
(15) Qinghaosu Antimalaria Coordinating Research Group, *Chin. Med. J.* **1979**, *92*, 811.
(16) Waki, H.-M.; Zhu, M.-Y.; Li, J.; Qian, Y.-L.; Li, G.-D. *Acta Pharm. Sinica* **1988**, *9*, 160.
(17) Li, G. Q.; et al. *Natl. Med. J. China* **1982**, *62*, 293.
(18) Myint, P. T.; Shwe, T. *Southeast Asian J. Trop. Med. Public Health* **1986**, *17*, 19.
(19) Thaithong, S.; Beale, G. H. *Bull. W.H.O.* **1985**, *63*, 617.
(20) Wang, T.; Xu, R. *J. Tradit. Chin. Med.* **1985**, *5*, 240.
(21) Wang, T.; et al. *J. Tradit. Chin. Med.* **1981**, *22*, 32.
(22) Win, K.; Thwe, Y.; Than, K.; Aung, A.; Tin, W. *Burm. Med. J.* **1985**, *31*, 26.
(23) Brossi, A.; Venugopalan, B.; Gerpe, L. D.; Yeh, H. J. C.; Flippen-Anderson, J. L.; Buchs, P.; Luo, X.-D.; Milhous, W.; Peters, W. *J. Med. Chem.* **1988**, *31*, 645.
(24) Yang, Q.; Shi, W.; Li, R.; Gan, J. *J. Tradit. Chin. Med.* **1982**, *2*, 99.
(25) Yang, S.-D.; Ma, J.-M.; Sun, J.-H.; Chen, D.-X.; Song, Z.-Y. *Chin. J. Clin. Pharmacol.* **1985**, *1*, 106.
(26) Li, C.-S.; Du, Y.-L. *Acta Pharm. Sinica* **1986**, *21*, 165.

Table I. Physical Properties of the Ester 6 and Acid 7 Derivatives of Dihydroartemisinin (2a)



compd	R	mp, °C	formula	TLC solvent	R_f	yield, %	anal.
6a	(*R*)-$CH_2CH(CH_3)COOCH_3$	63–65	$C_{20}H_{32}O_7$	EtOAc/Hex 1:2	0.8	60	C, H
6b	(*S*)-$CH_2CH(CH_3)COOCH_3$	oil	$C_{20}H_{32}O_7$	EtOAc/Hex 1:5	0.5	60	C, H
6c	(*S*)-$CH(CH_3)CH_2COOCH_3$	76–78	$C_{20}H_{32}O_7$	EtOAc/Hex 1:3	0.8	56	C, H
6d	(*R*)-$CH(CH_3)CH_2COOCH_3$	oil	$C_{20}H_{32}O_7$	EtOAc/Hex 1:4	0.5	70	C, H
6e	1-adamantylmethyl	145–147	$C_{26}H_{40}O_5$	EtOAc/Hex 1:4	0.8	60	C, H
7a	(*R*)-$CH_2CH(CH_3)COOH$	138–140	$C_{19}H_{30}O_7$	*a*		80	C, H
7b	(*S*)-$CH_2CH(CH_3)COOH$	118–120	$C_{19}H_{30}O_7$	*a*		90	C, H
7c	(*S*)-$CH(CH_3)CH_2COOH$	149–150	$C_{19}H_{30}O_7$	*a*		70	C, H
7d	(*R*)-$CH(CH_3)CH_2COOH$	gum	$C_{19}H_{30}O_7$			61	C, H

[a] Purified by recrystallization.

against *Plasmodium berghei* in mice.[2,27] Sodium artelinate possesses comparable antimalarial activity in vivo as well as in vitro to artemether or arteether. Because of its encouraging chemical and biological properties, **3** is currently being subjected to preclinical testing.

We report here in our attempt to find additional hydrolytically stable dihydroartemisinin derivatives with high antimalarial potency. A further goal in preparing this series was to examine the impact of stereospecificity of the introduced optically active ether side chains on therapeutic efficacy.

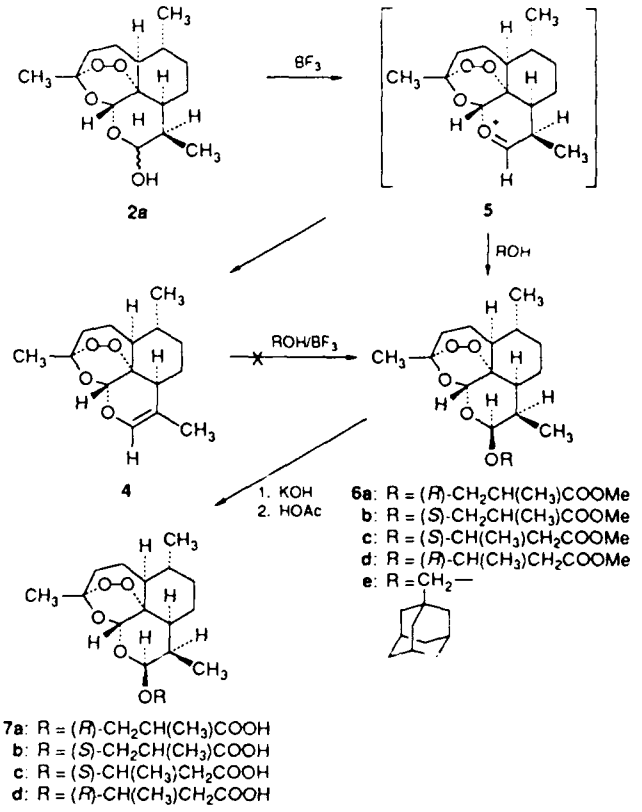
Chemistry

Dihydroartemisinin (**2a**) was prepared by sodium borohydride reduction of **1** according to a modified literature procedure[2,3] and was converted to the new ether derivatives by treatment of **2a** with an appropriate alcohol under boron trifluoride etherate catalysis at room temperature. The yields of the purified condensation products were 60–70% (Table I). Minor byproducts, artemether (**2b**) and 9,10-dehydrodeoxoartemisinin (**4**), were also isolated in some of the reactions. The formation of **2b** was discussed earlier.[2] It was observed that the yield of **4** increased when a secondary alcohol was used. For example, attempts to condense **2a** with methyl 3-hydroxy-4,4,4-trichlorobutyrate failed and gave, instead, a good yield of **4**. This observation suggests the involvement of the oxonium ion (**5**) as the common intermediate for the observed products (cf. Scheme I).

As the oxonium ion is a chemically reactive intermediate, either it reacted, once formed, with an alcohol to give the desired ether **6** or, in the absence of a nucleophile (alcohol), it tautomerized to **4**. Because both reactions compete for the same oxonium ion and the rate of reaction of the oxonium ion with the sterically hindered secondary or tertiary alcohols is slower than with the less hindered primary alcohols, tautomerization of the oxonium ion to 9,10-dehydrodeoxoartemisinin (**4**) became a predominant reaction. This may account for the good yield of **4** when the reaction was carried out with methyl 3-hydroxytrichlorobutyrate. Compound **4** has been reported to be the major product when triphenylmethanol was used.[28]

An alternative mechanism, which was considered, for the formation of the desired ether **6a–d** involves the reaction of the starting alcohol with 9,10-dehydrodeoxoartemisinin

Scheme I



(**4**); however, when purified **4** was treated with a primary alcohol under the catalysis of BF_3·Et_3, no reaction was observed. This result rules out the involvement of **4** as an intermediate in the formation of **6a–d**.

For a large-scale synthesis, compound **4** can be conveniently prepared in 75% yield by the treatment of **2a** with BF_3·Et_2O in ether solution at room temperature overnight. A long range coupling (J = ~3.6 Hz) between the newly formed olefinic proton and the methyl group on C-9 was observed.

The major ether condensation products **6** were obtained in 60–70% yields and were identified as the β isomers by their small coupling constants between H-9 and H-10 (J = 3.6–5.0 Hz) (see Table II). Saponification of **6** with 2.5% KOH/MeOH followed by acidification with cold dilute HCl gave the corresponding carboxylic acid **7** (Table I). The identity of all products were established by ^1H

(27) Lin, A. J. Data to be published.
(28) Li, Y.; Yu, P.; Chen, Y.; Li, L.; Gai, Y.; Wang, D.; Zheng, Y. *Acta Pharm. Sinica* **1981**, *16*, 429.

Table II. ^{1}H NMR Data of Compounds **6a-e** and **7a-d**

compd	chemical shifts, δ
6a	5.38 (s, 1 H), 4.76 (d, 1 H, J = 3.6 Hz), 3.95 (m, 1 H), 3.67 (s, 3 H), 3.43 (m, 1 H), 2.64 (m, 1 H), 1.43 (s, 3 H). 1.16 (d, 3 H, J = 7.2 Hz), 0.95 (d, 3 H, J = 6.3 Hz), 0.86 (d, 3 H, J = 8.1 Hz)
6b	5.38 (s, 1 H), 4.77 (d, 1 H, J = 3.6 Hz), 4.02 (m, 1 H), 3.66 (s, 1 H), 3.47 (m, 1 H), 2.65 (m, 1 H), 1.43 (s, 3 H), 1.21 (d, 3 H, J = 7.2 Hz), 0.94 (d, 3 H, J = 5.4 Hz), 0.87 (d, 3 H, J = 7.2 Hz)
6c	5.42 (s, 1 H), 4.90 (d, 1 H, J = 3.6 Hz), 4.37 (m, 1 H), 3.68 (s, 3 H), 1.43 (s, 3 H), 1.16 (d, 3 H, J = 6.3 Hz), 0.94 (d, 3 H, J = 7.2 Hz), 0.87 (d, 3 H, J = 7.2 Hz)
6d	5.42 (s, 1 H), 4.93 (d, 1 H, J = 4.5 Hz), 4.17 (m, 1 H), 3.66 (s, 3 H), 1.43 (s, 3 H), 1.33 (d, 3 H, J = 6.3 Hz), 0.95 (d, 3 H, J = 5.4 Hz), 0.82 (d, 3 H, J = 7.2 Hz)
6e	5.31 (s, 1 H), 4.68 (d, 1 H, J = 3.6 Hz), 3.45 (d, 1 H, J = 9.0 Hz), 2.83 (d, 1 H, J = 9.0 Hz), 1.39 (s, 3 H), 0.92 (d, 3 H, J = 2.7 Hz), 0.87 (d, 3 H, J = 7.2 Hz)
7a	8.32 (br s, 1 H), 5.42 (s, 1 H), 4.80 (d. 1 H, J = 3.6 Hz), 4.00 (m, 1 H), 3.51 (m, 1 H), 2.67 (m, 1 H), 1.44 (s, 3 H), 1.20 (d, 3 H, J = 8.1 Hz), 0.94 (d, 3 H, J = 3.6 Hz), 0.88 (d, 3 H, J = 7.2 Hz)
7b	7.41 (br s, 1 H), 5.41 (s, 1 H), 4.80 (d, 1 H, J = 3.6 Hz), 4.03 (m, 1 H), 3.49 (m, 1 H), 2.66 (m, 1 H), 1.44 (s, 3 H), 1.20 (d, 3 H, J = 7.2 Hz), 0.95 (d, 3 H, J = 4.5 Hz), 0.88 (d, 3 H, J = 7.2 Hz)
7b	7.41 (br s, 1 H), 5.41 (s, 1 H), 4.80 (d, 1 H, J = 3.6 Hz), 4.03 (m, 1 H), 3.49 (m, 1 H), 2.66 (m, 1 H), 1.44 (s, 3 H), 1.20 (d, 3 H, J = 7.2 Hz), 0.95 (d, 3 H, J = 4.5 Hz), 0.88 (d, 3 H, J = 7.2 Hz)
7c	7.98 (br s, 1 H), 5.40 (s, 1 H), 4.85 (d, 1 H, J = 4.5 Hz), 4.27 (m, 1 H), 1.36 (s, 3 H), 1.21 (d, 3 H, J = 5.4 Hz), 0.86 (d, 3 H, J = 3.6 Hz), 0.80 (d, 3 H, J = 7.2 Hz)
7d	8.03 (br s, 1 H), 5.43 (s, 1 H), 4.96 (d, 1 H, J = 3.6 Hz), 4.20 (m, 1 H), 1.43 (s, 3 H), 1.35 (d, 3 H, J = 6.3 Hz), 0.95 (d, 3 H, J = 5.4 Hz), 0.83 (d, 3 H, J = 7.2 Hz)

Table III. In Vitro Antimalarial Activities against *P. falciparum*

no.	IC_{50}, ng/mL	
	Sierra Leone (D-6)	Indochina (W-2)
1	2.35	2.60
2a	0.41	0.69
3	4.07	1.38
4	0.83	0.43
6a	2.15	0.057
6b	0.36	0.015
6c	1.91	0.48
6d	0.44	0.071
6e	2.70	2.11
7a	22.24	5.32
7b	28.72	8.33
7c	15.04	7.43
7d	36.47	4.42
chloroquine	3.1	43.60
quinine	3.61	59.00

NMR (Table II) and IR spectrometry and elemental analysis.

Results and Discussion

The water-soluble dihydroartemisinin derivatives and their ester precursors were tested in vitro against human malaria parasite, *P. falciparum* D-6 (Sierra Leone clone) and W-2 (Indochina clone). The former clone is a strain that is resistant to mefloquine, and the latter, to chloroquine, pyrimethamine, sulfadoxine, and quinine.

The in vitro test results indicate that the new derivatives, like the parent agents **1** and **2a**, are not cross-resistant to any of the existing antimalarial agents (Table III). The derivatives are, in general, more effective against the W-2 than the D-6 strain. The esters (**6a–d**) possess superior activity to that of the parent compound **1**, arteether, and artemether. Conversion of the esters to their corresponding carboxylic acids drastically reduced their antimalarial activity by 10–100-fold and none of the latter possess superior antimalarial activity to artelinic acid (**3**). The olefinic compound 9,10-dehydrodeoxoartemisinin (**4**) is more active than artemisinin and is as active as dihydroartemisinin (**2a**) (Table III).

Table IV. Antimalarial Activity of Dihydroartemisinin Derivatives against *P. berghei* in Mice

compd	dosage, mg/kg per day	no. of mice cured[a]
control	0	0/5
1	640 × 3	5/5
	160 × 3	5/5
	80 × 3	2/5, 3/5 (A)
	20 × 3	0/5, 2/5 (A)
4	640 × 3	4/5, 1/5 (A)
	160 × 3	1/5, 3/5 (A)
	80 × 3	2/5, 1/5 (A)
	20 × 3	0/5
6a	320 × 3	5/5
	80 × 3	1/5, 3/5 (A)
	20 × 3	0/5, 2/5 (A)
6c	320 × 3	5/5
	80 × 3	5/5
	20 × 3	1/5, 4/5 (A)
6e	320 × 3	3/5
	80 × 3	0/5, 5/5 (A)
	20 × 3	0/5

[a] The terms cure and active (A) are defined in the Experimental Section.

Significant stereospecificity was observed among the esters **6a–d**; however, no difference was seen among the acids **7a–d**. Between the two esters possessing the 2-methylpropionate side chain, **6b** is 4 times more effective than **6a** against W-2 and is 7 times more active against D-6. Likewise, compounds **6c** and **6d** possessing the 3-methylpropionate side chains showed stereospecificity. Compound **6d** is about 5–7 times more effective than **6c**. The results suggest that oxidative dealkylation of the alkyl side chain may be a prerequisite for the antimalarial activity.

The superior antimalarial activity of the esters over the acids suggest that lipophilicity may play an important role in determining the antimalarial activity of this series. Since adamantyl function is known for its high lipophilicity, compound **6e** was prepared and tested side by side with esters to examine whether high lipophilicity, per se, will contribute to higher activity. Although compound **6e** with a lipophilic adamantyl function was expected to be more lipophilic than **6a–d**, its antimalarial activity is, nevertheless, 5–40 times lower than that of **6a–d**.

Since the in vitro antimalarial activity of the acids are weak and the number of ester derivatives synthesized was limited, only three compounds, **6a**, **6c**, and **6e**, were selected for further testing in mice (cf. Table IV and Figure 1). Compound **6c** was the most active of the three esters inasmuch as the mice that received either 320 or 80 mg/kg per day (MKD) were judged to be cured (blood film negative for 60 days). Of the five mice that received 20 MKD, only one was cured; however, the other four mice exhibited extended survival times. Compound **6a** was the second most active compound with 5/5 mice receiving 320 MKD being cured. Only one mouse was cured at 80 MKD, whereas, the remaining four had extended survival times (16.8 days). No mice were cured at the lower dose of 20 MKD; however, they all had extended survival times. Compound **6e** was the least active compound with only three mice cured at 320 MKD. The remaining mice did not have extended survival times. Toxicity was observed in all the mice at 320 MKD and marked skin lesions occurred at the site of drug injection. These lesions persisted for the duration of the 60 days. Mice receiving 80 MKD

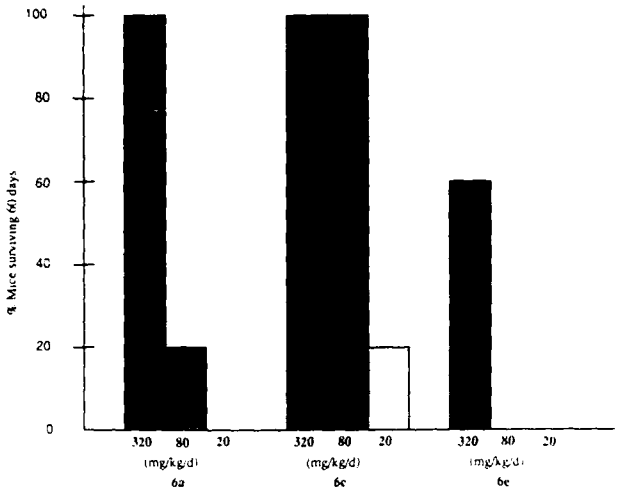


Figure 1. Antimalarial activity of three dihydroartemisinin derivatives: **6a**, **6c**, and **6e**.

had extended survival (15.8 days) but no cures, whereas 4/5 mice in the 20 MKD group did not have extended survival times.

When these three new dihydroartemisinin analogues were compared with artemisinin itself, only **6c** was more active at the 80 MKD level. Artemisinin had only two cures, whereas all five mice in this experiment were cured with **6c**.

Since dihydroartemisinin (**2a**) is a potent antimalarial agent, one of the major factors in determining the efficacy of its derivatives may be the facility of oxidative dealkylation of the introduced side chain in the parasites or the host liver cells to regenerate the dihydroartemisinin. The overall inferior antimalarial activity of the acid relative to the ester derivatives of dihydroartemisinin suggests the requirement of a high degree of lipophilicity for a good antimalarial potency. Thus far, the sodium salt of artelinic acid remains the most active of the water-soluble derivatives of **2a** that we have prepared.

Experimental Section

Chemistry. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra of solid samples were obtained in KBr disks on a Perkin-Elmer Model 283 spectrophotometer or Nicolet 20SXB FT-IR spectrometer. NMR spectra were run on a JEOL FX90Q spectrometer using Me_4Si as an internal standard. Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI, and the results were within 0.4% of the expected values, except where noted. Chromatotron Model 7924 T is manufactured by Harrison Research, 840 Moana Court, Palo Alto, CA.

Condensation of Dihydroartemisinin (2a) with Alcohols. Dihydroartemisinin[2] (**2a**, 0.5 g, 1.75 mmol) was dissolved in 70 mL of anhydrous Et_2O. To the solution was added 5 mmol (excess) of an appropriate alcohol, followed by 0.25 mL of $BF_3{\cdot}Et_2O$. The reaction mixture was stirred at room temperature for 24 h, washed successively with 5% aqueous $NaHCO_3$ and H_2O, dried over $MgSO_4$, and evaporated to dryness under reduced pressure. The resultant oil was purified by preparative TLC or by a Chromatotron TLC device, using EtOAc/hexane as the eluant (Table I). The ^1H NMR data are as expected (Table II). The minor products **2b** and **4** were also isolated from the reaction mixtures of **6c** and **6d**.

Conversion of Esters 6a–d to the Corresponding Carboxylic Acids 7a–d. Ester **6** (1 g) was dissolved in 30 mL of 2.5% KOH/MeOH and allowed to stand at room temperature for 2 days. To the solution, an equal volume of H_2O (30 mL) was added and the MeOH was removed under reduced pressure. Upon cooling in an ice bath, the aqueous solution was acidified with dilute HCl and extracted 3 times with Et_2O. The Et_2O extracts were combined, dried over $MgSO_4$, and evaporated to dryness. The residue was recrystallized from an appropriate solvent (Table I).

9,10-Dehydrodeoxoartemisinin (4). To dihydroartemisinin (5 g) in 500 mL of anhydrous ether was added 0.5 mL of $BF_3{\cdot}Et_2O$. The solution was stirred at room temperature overnight, washed successively with 5% aqueous $NaHCO_3$ and H_2O, dried over $MgSO_4$, and evaporated to dryness. The resultant crystals were recrystallized from petroleum ether to give 4 g (85%) of 9,10-dehydrodeoxoartemisinin (**4**): mp 95–97 °C (lit.[28] mp 96–98 °C); NMR ($CDCl_3$) δ 6.19 (br s, 1 H), 5.54 (s, 1 H), 1.58 (d, J = 0.9 Hz, 3 H), 1.42 (s, 3 H), 0.99 (d, J = 3.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$) ppm 16.11, 20.17, 24.34, 25.75, 29.92, 34.04, 36.15, 37.40, 44.39, 51.38, 78.84, 89.58, 104.41, 107.93, 134.91.

Biology. (a) In Vitro Antimalarial Studies. The in vitro assays were conducted by using a modification of the semiautomated microdilution technique of Desjardins et al.[29] and Milhous et al.[30] Two *P. falciparum* malaria parasite clones, designated as Indochina (W-2) and Sierra Leone (D-6), were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation from patient isolates obtained by the Centers for Disease Control, Atlanta, GA, in 1980 and 1982, respectively. The patients had acquired infections either in Vietnam or Sierra Leone. The Indochina clone is resistant to the antimalarials chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the Sierra Leone is resistant to mefloquine but susceptible to chloroquine, quinine, sulfadoxine, and pyrimethamine. Test compounds were initially dissolved in DMSO and 70% ethanol and diluted in RPMI 1640 culture medium with 10% human plasma to 400-fold. These solutions were subsequently further diluted with the Cetus Pro/Pette (Perkin-Elmer Corp., Norwalk, CT) over a range of (1.56–100) $\times$ 10^{-9} M. Parasite inocula (at 0.5% parasitemia and a 1% hematocrit) were incubated for 24 h and added to equimolar concentrations of each test compound prior to the addition of [^{3}H]hypoxanthine. After a further incubation of 18 h, particulate matter was harvested from each microtiter well using an automated cell harvester (Skatron, Inc., Sterling, VA). Uptake of [^{3}H]hypoxanthine was measured with a scintillation spectrophotometer (Model LS3801, Beckman Instruments, Irvine, CA). Concentration–response data were analyzed by nonlinear regression and the IC_{50} values (50% inhibitory concentrations) for each compound were calculated.

(b) In Vivo Antimalarial Studies. The suppressive blood schizonticidal and curative activities of test compounds (**6a,c,e**) were determined in a test where mice were infected with 5.98 $\times$ 10^5 *P. berghei* parasitized cells intraperitoneally on day 0. Test compounds were dissolved in peanut oil and were administered subcutaneously once a day for 3 consecutive days commencing on day 3. The dose levels of compounds given were 320, 80, and 20 mg/kg per day. Blood films were taken on day 6 and then every 7 days until day 60 Blood schizonticidal activity was determined by monitoring blood films for the appearance of parasites and for extended survival times compared to infected untreated controls. Mice surviving 60 days were considered cured. The infected untreated control mice (negative controls) died on either day 6 or 7. Compounds were considered active when the survival time of the treated mice was greater than twice the control mice, i.e., 12–14 days.

Acknowledgment. We thank Dr. Arba L. Ager, Jr., University of Miami, for performing the in vivo and Dr. Wilbur K. Milhous for performing the in vitro antimalarial studies.

Registry No. α-**2a**, 81496-81-3; β-**2a**, 71939-50-9; **2b**, 71963-77-4; **4**, 82596-30-3; **5**, 119970-58-0; **6a**, 119946-72-4; **6b**, 120020-65-7; **6c**, 119946-74-6; **6d**, 120020-67-9; **6e**, 119946-76-8; **7a**, 119946-73-5; **7b** 120020-66-8; **7c**, 119946-75-7; **7d**, 120020-68-0; (*R*)-$HOCH_2CH(CH_3)COOMe$, 72657-23-9; (*S*)-$HOCH_2CH(CH_3)COOMe$, 80657-57-4; (*R*)-$HOCH(CH_3)CH_2COOMe$, 3976-69-0; (*S*)-$HOCH(CH_3)CH_2COOMe$, 53562-86-0; 1-adamantylmethanol, 770-71-8.

(29) Desjardins, R. E.; Canfield, C. J.; Haynes, D. E.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
(30) Milhous, W. K.; Weatherley, N. F.; Bowdre, J. H.; Desjardins, R. E. *Antimicrob. Agents Chemother.* **1985**, *27*, 525.